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April 7, 1999.

Lyndon Hawkins
CALEPA-DPR
Environmental Monitoring and Pest Management
1020 N. Street, Room 161
Sacramento CA 95814-5624

Dear Mr. Hawkins,

Please find enclosed the final report for our project entitled "Mating Disruption of Carob Moth in Dates", DPR contract 95-0247. I apologise for its being slightly late; it was held up while I double-checked some of the factual information with my collaborators in the date industry. Please let me know if you need any further information; my email is jocelyn.millar@ucr.edu.

Yours sincerely,

Jocelyn G. Millar
Professor

CALIFORNIA DEPARTMENT OF PESTICIDE REGULATION

PROJECT REPORT, 1998

PRINCIPAL INVESTIGATORS: Jocelyn G. Millar and Harry H. Shorey (deceased)
Dept. of Entomology, University of California
Riverside CA 92521

PROJECT TITLE: MATING DISRUPTION OF CAROB MOTH IN DATES

SUMMARY:

This was our second attempt to control carob moth by pheromone-mediated disruption of mating using aerosol cans of pheromone fitted with timed release mechanisms. The project was delayed a year because of the failure of several custom chemical manufacturers to deliver the pheromone chemical. Furthermore, once a supplier was finally found who could make the pheromone, the product was highly impure. Contrary to the 1996 trials using pheromone made at UC Riverside, this impure commercial pheromone resulted in poor trap shutdown, with significant numbers of male moths being attracted to traps in treated areas seasonlong, despite numerous efforts to identify the problem(s) and improve the efficacy of the communication disruption. The project suffered another disastrous blow when Dr. Harry Shorey and his field assistant were tragically killed in a traffic accident while servicing project field sites. Every effort was made to continue the project and identify and remedy the problems, but moth infestation reached levels that required the larger of the two field sites to be treated with insecticide and the pheromone-only treated area of the smaller of the two sites was decreased to minimize loss of the date crop. There was considerable evidence that the control failures were due in large part to poor quality commercial pheromone, and further contamination during formulation. Further studies are on hold, pending the commercial scaleup of a simpler synthesis of the pheromone material, and field tests of the attractiveness of this new material to male moths before going ahead with mating disruption trials. Nevertheless, the date industry is committed to the continued development of this technology.

RESULTS AND DISCUSSION

1. Overview of Experiment:

This project tested mating disruption of carob moth, the principal insect pest of dates in California, using a mimic of the natural pheromone of the moth (hereafter referred to as pheromone for simplicity). Two plots were used, a 40 acre block at Hadley Date Gardens, off Airport Blvd. west of Thermal airport, and a block of 130 acres plus 30 acres of date offshoots and citrus just north of the Salton Sea, near the intersection of highways 86 and 195 (Rancho Eileen). Both sites had mostly medium-height trees (20-40 feet) on ~30 foot spacing. The 40 acre block was the same one used in the 1996 trials. Maps of the two sites are attached (Fig. 1). In addition to the pheromone treatment, most of each block was treated with malathion dust for carob moth control, with an "island" of several acres treated only with pheromone in the center.

Pheromone release devices (puffers) were put in place on July 2, placed on every second tree around the entire perimeter of each of the two blocks, including around the part of the 160 acre block consisting of offshoots and citrus, giving approx. 60 foot spacing between puffers. For the 160 acre block, puffers were also placed at the same spacing along the vertical axis of the block, and along one horizontal at the large end of the "dates-only" 130 acre part of the block. Puffers were placed alternately at the base of the crown and at half-height. If trees were missing, the puffer was placed on the tree in that position in the next row in. Puffers were placed on both male and female trees. In total, 88 puffers were placed around the 40 acre block, and 264 puffers were placed in and around the 160 acre block. Puffers were suspended from fronds and axils with large metal hooks, approx. 6 in in diameter. The hooks were constructed with a straight shaft which could be inserted in the end of a mounting pole, so that they could be hung at heights of 15-20 ft from the ground. These ones were also easily taken down for checking with the same pole device. Higher-up puffers were placed by ladder men or a cherry picker. Dr. Shorey and his assistant had just finished replacing the pheromone reservoirs on August 17 when they were killed in a traffic accident.

The puffer devices consisted of a custom-built circuit board, programmable with an infrared remote control, which controlled a solenoid valve, all powered by 4 AA cell batteries, and contained within a plastic cabinet with removable back. The valve released

metered puffs of 30 microliters/puff, with each puff containing 0.2 mg pheromone. The initial program was set so that puffs were released every 15 minutes from 6:00 P.M. to 6:00 AM the next morning. The puffer devices were made to be checkable from the ground with the hand-held infrared remote control, i.e., there was interactive circuitry which could report the status of each puffer to the handheld remote control, allowing for easy checking of the status of each puffer, as long as the puffer was within range of the remote.

The single most important factor which has hindered progress on this project has been the difficulty in finding a chemical manufacturer with the capability of making the pheromone component in kilogram scale, in sufficient purity for use. Since the inception of this project, we have contracted with five different companies to produce a kilo of the material. The first company was not able to finish the synthesis, and sent the half-finished material to UCR to be completed in Millar's laboratories. This material was used in the promising field trials in 1996. The next three companies failed completely, and the final company, Chemica Technologies of Bend OR, finally produced the required quantity of material, but only after long delays and substantial budget overruns, and the product was heavily contaminated with impurities. Despite the contract with Chemica Technologies being placed many months in advance, the first portion of pheromone was delivered at the end of June 1998, only a few days before we would have had to postpone the project for a second year. Because only a portion of the pheromone was available by the start date, two sets of puffer reservoirs were used. The first set were loaded (by Roland Gerber at Kearney Ag. Center) with an 83 day charge in aerosol cans, consisting of about 1 gram of pheromone diluted with acetone and hexane, and stablized with BHT. The rest of the charge consisted of the propellant Dymel 134a (Dupont). The second set was loaded with the remainder of the pheromone material, formulated for a 90 day charge, as soon as it became available. The second batch of pheromone was of very poor quality (~66% pure), but at that point there was no choice: we had to use this material, or scrap the experiment.

The pheromone-treated blocks and a control block were monitored with traps baited with 11 mm grey rubber septa (The West Co., Lititz PA) loaded with 0.5 mg of pheromone mimic (standard solution prepared by Millar May 16, 1992, and used for baits

in trials to date). The 160 acre block at Rancho Eileen had a grid of 12 traps, whereas the 40 acre Hadley block was monitored with six traps. A single trap placed in each of seven standard-practices date gardens provided data for pheromone-free controls. Traps used were standard Pherocon 1C sticky traps, and they were monitored weekly from the beginning of July 1998 through the middle of January, 1999. Traps were hung at the crowns of trees using a rope and eye, flagpole-type system as done in previous years.

2. Field experiments:

Shortly after initiating the mating disruption experiment on July 2, it became clear that the pheromone treatments were not completely effective, as shown by significant monitoring trap captures in the treated blocks (Figs 1 and 2). Through July and into August, the project field supervisor (Mr. John Davies) and Dr. Shorey made numerous attempts to troubleshoot and improve the performance of the puffer devices, as follows:

1. The puffer devices at each site were checked to make sure that they were releasing pheromone properly. The failure rate was determined to be less than 10%, effectively eliminating mechanical problems as a cause of the failure. As a further check, the pheromone reservoirs were weighed, and it was obvious from the weight losses that pheromone was indeed being dispensed at the correct rates.
2. The heights of the puffers were altered, placing them on a low (chest-height), medium (half tree height) and high (under crown) spacing, to try and provide uniform coverage of the entire airspace in the blocks. An experiment was also run with live females and synthetic pheromone lures as trap baits, with traps placed at the top, middle and bottom of trees. Overall trap catches with both females and lures were low, but male moths were caught at all three heights, suggesting that blanketing the entire airspace of the date garden is probably desirable.
3. The puffers were set to dispense pheromone 24 hours a day, instead of only from 18:00 to 06:00 the next morning, in case puffing from 18:00 on did not saturate the sites with pheromone. Furthermore, a check of moth activity periods was made, using live females as bait. As previously reported, female calling behavior peaked around midnight, so even with puffers switching on only at 18:00, there should still have been ample time for saturation of the date garden atmosphere with pheromone.

4. The first batch of commercial pheromone was reanalyzed by coupled gas chromatography-mass spectrometry, but no unexpected or unknown compounds were found at significant levels. Furthermore, a check of a subsample of used pheromone reservoirs recovered from the field determined that the pheromone was not degrading significantly in the pheromone reservoirs recovered from the field, indicating that the metal reservoir was protecting the pheromone from air and light as anticipated.
5. All the remaining pheromone on hand was loaded into puffers, and these extra puffers were deployed around and through the middle of each block, providing a density of ~ 2 puffers/acre.
6. Because the first batch of puffers had been shifted to 24 hour operation, instead of 12 hour operation as planned, their pheromone charges were exhausted well in advance of their projected 83 day field lifetimes. Consequently, the puffer reservoirs were changed on August 17, instead of the originally planned date of about the middle of September.
7. An experiment using live female moths as trap baits was conducted in pheromone treated and control blocks (3 traps/treatment, counted twice). Although trap catches of male moths were reduced in the pheromone treated blocks (2.8 ± 4.1 moths/trap) versus control blocks (15.7 ± 15.9 moths/trap), they were not completely eliminated, again indicating incomplete disruption of female-male moth communication.

However, all attempts to reduce male moth trap captures further in the treated blocks were to no avail, and captures continued at unacceptably high levels. To minimize damage to the crop, at the beginning of September, the experiment at Rancho Eileen was stopped, and the pheromone-only "island" in the center of the block was treated with malathion to minimize further crop loss. Monitoring with pheromone traps was continued until after harvest in mid-January.

To try and salvage the experiment in the smaller block at the Hadley Ranch, and to try and determine whether the poor results were due to the pheromone or to the dispensing method, at the beginning of September the block was treated with hollow fiber type dispensers left over from trials in 1995. These fibers contained pheromone made at

UC Riverside. Six fiber dispensers were stapled to every second tree throughout the block, but trap catches still remained high.

Despite the potential for crop loss, our collaborator Albert Keck at the Hadley Ranch decided to continue the experiment until harvest, which was very late in 1998, with the last dates not being harvested until the middle of January, 1999. Periodic checks of the puffer dispensers were continued, and it was found that the puffer mechanisms were performing as expected, with a failure rate of <10%. Thus, mechanical failure can be ruled out as the cause of the incomplete trap shutdown.

3. Infestation levels at harvest.

Samples of approx. 1000 dates (10 x 100 dates/site) were checked for infestation at harvest, from both the pheromone-only and the malathion + pheromone areas of the experimental blocks. In addition, we were able to obtain analogous data from three commercial growers using standard malathion treatments, and further data obtained during the grading of the dates from the Hadley site during processing for sale. The data are shown in Table 1. Infestation levels (~4 %) in the pheromone-only samples from the Hadley site were slightly higher but not significantly different from the malathion plus pheromone treatments, and were in the same range as the other infestation levels from the cooperating grower sites (0.6-2.36% infestation). Furthermore, these levels of 4% are well within industry standards; in some years, infestation levels average over 10%.

Table 1. Percent infestation of dates with carob moth at harvest.

Site	Treatment*	# samples	% Infestation (mean ±SD)
Experiment:			
Hadley	M + P	9	2.44 ± 1.42
Hadley	M + P	9	2.90 ± 3.03
Hadley	P	9	4.11 ± 3.58
Rancho Eileen**	M + P	12	4.92 ± 3.32
Other Cooperators:			
Alamo	M	11	2.36 ± 1.36
NW block	M	8	1.75 ± 1.83
Negosian	M	10	0.6 ± 0.97
Commercial grading:			
Hadley	M + P	29	2.07 ± 2.10
Hadley	P	3	4.00 ± 2.60

* M = malathion treated; P = pheromone treated.

** The experiment was stopped at the Rancho Eileen sites in September, but samples were taken anyway to determine levels of infestation at harvest.

4. Testing and analysis of pheromone used in disruption trials.

Because of the seasonlong problems with incomplete trap shutdown, we reexamined the commercial pheromone in several different ways, as follows:

1. Because of concern that the commercial pheromone might not be as effective/attractive as the material made at UCR in previous years, a direct test was carried out. A sample of commercial pheromone was recovered from several puffer cans, and tested as a trap bait versus an equivalent dose of the UCR chemical (6 replicates). Traps baited with the UCR material caught significantly more moths (17.2 ± 12.9) than traps baited with the puffer chemical (2.0 ± 1.4; significantly

different by t-test), clearly indicating that the material recovered from the puffer cans was inferior.

2. A subsequent test was conducted to determine whether the commercial material contained repellent/inhibitory impurities, or whether it was not as attractive as the UCR material for other reasons. Thus, the UCR material was tested versus a 1:1 mixture of the UCR material with the commercial material, and versus the commercial material alone. Each treatment was replicated 4 times, and counted twice. Traps baited with the UCR chemical caught an average of 111 ± 53 moths, the mixture caught 32 ± 25 moths, and the commercial chemical alone caught 8 ± 2 moths, strongly suggesting that the commercial material was contaminated with an inhibitory impurity (UCR chemical significantly better than the mixture or the puffer pheromone; oneway ANOVA and Student-Neumann-Kuels test).

   This finding was verified by fractionating the commercial material recovered from a puffer reservoir by liquid chromatography on silica, taking five fractions. The second fraction, containing the purified pheromone component, was then remixed with each of the other 4 fractions in binary combinations, and these blends were tested as attractants in traps (Table 2). Because the test was set out late in the season, overall trap catches were low and variable, and the treatments were not statistically different due to the large standard deviations. Nevertheless, it seems fairly clear that of all the fractions, the first fraction would be the most likely to contain the inhibitory components.

Table 2. Carob moths caught in traps baited with fractions of the commercially produced carob moth pheromone.

Fraction #	Moths caught (Mean ±SD)
2 (purified pheromone)	7.5 ± 4.3
2 + 1	0.7 ± 1.2
2 + 3	4.0 ± 2.5
2 + 4	3.7 ±4.0
2 + 5	4.8 ± 4.4

As a further demonstration that the material recovered from the puffer cans contained inhibitory compounds which were being detected by the moths, we also conducted coupled gas chromatography-electroantennogram detection studies on the material. This technique uses a gas chromatograph to separate an extract into its individual components, which are then passed over a live insect antenna hooked to an amplifer. Compounds which are detected by the insect antenna generate a voltage spike as the antennal receptors are stimulated, and by matching up this signal with the corresponding signal from the gas chromatograph's detector, it is possible to locate potentially inhibitory compounds in an extract. Using this technique, we verified that the material recovered from the puffer can did indeed contain several impurities which elicited signals from male carob moth antennae.

The inhibitory first fraction was analyzed by coupled gas chromatography-mass spectrometry, and was found to contain numerous compounds which would have taken months of iterative fractionations and bioassays to finally locate the inhibitory compound(s). Because of the limited budget and manpower, the decision was made to not pursue the identification of the inhibitory compound(s) at this time.

However, during the fractionation and analysis, it was noticed that the material recovered from the puffer reservoirs contained several components which were not in the material as received from Chemica Technologies. These compounds were identified as components of the peach twig borer and omnivorous leafroller pheromones, which must have been introduced into the pheromone reservoirs during filling. Because the pheromone reservoirs are aerosol cans which must be loaded under pressure with the gaseous propellant, a special pressurized loading system is required. Despite being thoroughly rinsed with solvent between fillings, the system apparently remained contaminated with pheromones from previous loadings. Consequently, another field test was carried out, testing samples from two batches of unused commercial pheromone, material recovered from a puffer can, and standard UCR material (Table 3).

Table 3. Moths caught in traps baited with commercial carob moth pheromone as received from the manufacturer, with commercial material after formulation and loading in puffer cans, and with UCR standard material (3 traps/treatment, counted twice).

Attractant	Moths caught (Mean ±SD)
UCR standard	14.2 ± 6.8
Commercial batch 1	10.0 ± 14.6
Commercial batch 2	5.2 ± 4.5
Commercial, recovered from puffer	0.33 ± 0.82

As was the case in a previous test, the large standard deviations obtained in trap catches in these late season tests obscured any significant differences between treatments. Nevertheless, the results suggest that the contamination causing the poor attractiveness of the commercial material is due in large part to impurities introduced during formulation and loading of the material, or due to the formation of inhibitory materials in the reservoirs exposed to field conditions. Investigations were halted at this point because it was too late in the season (mid-December) to conduct further bioassays due to declining moth populations.

Analysis of Possible Reasons for Failure of the Mating Disruption Trials

I. Pheromone.

a. Pheromone quality. Obtaining good quality pheromone has been the single biggest problem for this project. In the past, Millar has made numerous batches of pheromone in quantities >100 grams for this project, but the Millar lab has neither the manpower nor the facilities to make kilo quantities, nor is it the mandate of university laboratories to be doing this type of work. However, our experience with commercial laboratories has been both abyssmal and expensive; the date industry has lost considerable money on failed contracts with custom chemical manufacturers, several of whom have been outside the US, and so beyond reach of legal redress. After several years of these problems, we contracted with Chemica Technologies, and they did indeed produce the required amount of material, albeit late, and of very poor quality (the second batch was only about 66%

pure). At this point, the only solution seems to be the development of an easier, alternate synthesis for the compound, that will be easier for commercial manufacturers to carry out on large scale. Such a synthesis is under development, and we hope to have a sample of material made by this new process for testing within the next few weeks.

We are also reasonably sure that degradation of the active ingredient of the pheromone inside the puffer cans is not the cause of failure because we have repeatedly analyzed samples of materials recovered from field-aged pheromone reservoirs, and found consistently high titers of the active ingredient. However, as shown by the data above, contamination of the pheromone during loading into the pheromone reservoirs contributed substantially to the decrease in attractiveness of the pheromone material. This problem was not foreseen because the loading equipment had been used successfully for loading pheromone reservoirs for a number of other insects under study by Dr. Shorey's group. As populations of carob moths increase in the field this spring, we will carry out further tests with the identified contaminants (pheromones for peach twig borer and omnivorous leaf roller) to determine whether these contaminants might be the cause of the minimal attractiveness of the material recovered from the puffer cans. In future, we will also make every effort to retest pheromone (both in the laboratory and in field tests), after it has been loaded into puffer reservoirs but before the puffers are deployed, to try and forestall these type of problems. This past year, because of the very tight time line, this option was not open to us; as soon as we received the pheromone, it had to be formulated and deployed.

b. Pheromone quantity. In 1996, we used about 2 grams active ingredient per acre seasonlong, and during the first part of the season, before mechnical and other difficulties caused problems, we achieved good trap shutdown with this application rate. In 1998, we doubled this release rate to more than 4 grams active ingredient per acre. Contrary to our expectations, even with the increased release rate in 1998, trap shutdown was not as good as in 1996. This suggested that factors other than the release rate were contributing to the poor control, such as poor pheromone quality (i.e., the male moths were able to pick out the "true" blend produced by female moths against the background of pheromone being released from the dispensers) or incomplete blanketing of the date gardens with

pheromone (a dispenser problem, discussed below). Consequently, we are still unable to state explicitly what the recommended release rate of active ingredient should be.

II. Puffer devices

The mechanism and operation of the puffer devices was unlikely to have contributed to the failure of the experiments because tests throughout the season (producing test puffs, plus continued weight loss of the reservoirs indicating that pheromone was being dispensed) determined a low failure rate for the machines. This is in contrast to the 1996 season, when one of the most likely causes of the breakdown of control later in the season was mechanical problems with the release devices. Thus, we have every reason to believe that the puffers operated as designed, and that operation of the puffers was not a significant factor in the failure of the mating disruption experiments.

It must also be mentioned that the puffer devices or other large-scale point sources deployed at a density of only one or two per acre may not be the most effective way of saturating the atmosphere of a date garden with pheromone (see section below on 'Puffer Density" for further details). Further testing with good quality pheromone may reveal that a much larger number of small point sources perform more effectively. We originally began mating disruption trials using such a strategy, but it was temporarily shelved because of the cost and difficulties in manufacturing and deploying the large numbers of point sources required.

III. Experimental Design and Execution

a. Field Plots. The "island" design, with a pheromone-only island inside a larger block treated with both pheromone and malathion, seems to be the ideal setup for conducting mating disruption trials. This design has the advantages that only a small part of the overall plot is at risk with the experimental treatment, and the effects of mated moths migrating into the pheromone-only portion of the plot is minimized by the surrounding area treated with both malathion and pheromone. It has the further advantage that the pheromone-only island should be continuously blanketed with pheromone, no matter which way the wind is blowing because it is surrounded on all sides by a buffer zone with pheromone dispensers.

b. Puffer Density. We had originally planned on about 1 puffer per acre, a standard density which has worked well in fruit and nut crops. However, to be conservative, trials were started with a density of about 1.5 puffers/acre, and this was increased to about 2 puffers/acre when it became clear that trap shutdown was not being achieved. This used up all the available pheromone and puffer devices. Furthermore, increasing puffer density beyond about 2 puffers per acre becomes costly; if control cannot be achieved with this density of puffers, then mating disruption using these release devices may not be economically feasible for growers.

It must be pointed out that date gardens have several peculiarities that may make the puffer technology more difficult to implement that in other crops. A date garden essentially consists of a series of widely spaced (30 foot spacing) poles, with a tuft of foliage at the top. There is usually some weedy groundcover, but there is essentially no foliage between about waist height and the crowns of the trees. Consequently, unlike other tree crops, there is little foliage to retain and rerelease the pheromone, and most of the pheromone released may be blown out of the treated area rapidly. However, if this were the case, then we should not have achieved the almost complete trap shutdown that occurred during the first part of the 1996 trials using puffer release devices. Thus, there is at least a strong suggestion from the 1996 data that the puffer technology can work in dates if problems related to the pheromone material can be solved.

c. Puffer Placement. Puffers were initially placed just under the crown and at midheight. A couple of weeks later, further puffers were added at chest height, to try and ensure that the entire airspace of the treated gardens would be blanketed with pheromone. Some puffers were aimed to fire out into the orchard, to provide concentrated plumes of pheromone drifting through the date gardens, while others were turned towards the tree, so that they sprayed pheromone onto the bark, so that the tree trunk itself could act as a slow release device. At this point, we have no further suggestions for improving release device placement.

d. Deployment date. Puffers were deployed in early July, 1-2 carob moth generations before the dates become susceptible. Delays in the delivery of the pheromone material prevented earlier deployment. In future trials, every effort will be made to deploy the pheromone at least one month earlier, for two reasons. First, in the best case, starting one

generation earlier will help to drive carob moth populations even lower. Second, in the worst case, if control is not achieved, trap catches will show this and measures can be taken to minimize crop loss before the crop becomes susceptible to infestation.

e. Puff Intervals. In the 1998 trials, the puff interval was conservatively set at one puff every 15 minutes, a rate which has proven satisfactory for other crops. We started the experiment with all puffers set to fire every 15 min, 12 hours a day. This was switched to 24 hours per day after a few weeks, in an effort to get more complete trap shutdown. As with the release rate, it is not yet possible to state with any certainty that a 12 hour per day release period bracketing the hours of moth activity might be sufficient, because of the problems with the pheromone material itself.

Summary:

A large scale experiment attempting to control carob moth by pheromone-based mating disruption was not successful. The most likely cause of failure was the poor quality of the pheromone, exacerbated by inhibitory contaminants which were accidentally introduced into the pheromone during formulation. In contrast, the puffer release technology used to dispense the pheromone performed as expected. At this point, it is not clear whether factors other than poor pheromone quality (e.g., quantity of pheromone/acre, efficacy of the release technology, number of pheromone sources/acre) contributed to the control failures, although data from previous years suggests that this is not the case. The date industry is committed to pursuing the development of this technology, and in 1999, is funding work on the development of a new, more robust synthesis of the active ingredient in preparation for further field trials in 2000.

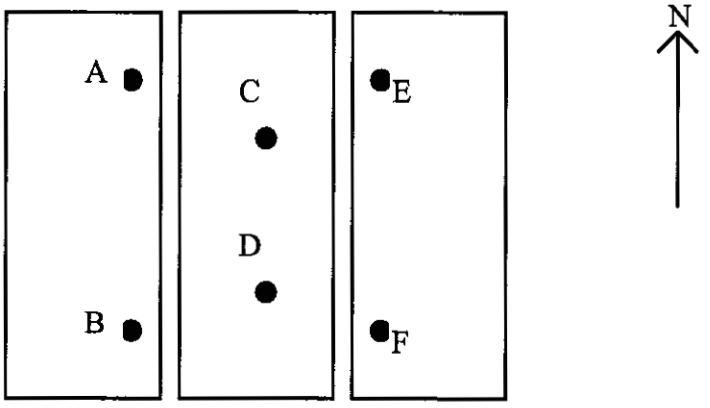
Acknowledgments.

This project could not have been accomplished without the hard work and persistence of the project field manager, John Davies, and Steve McElfresh and Roland Gerber, who pitched in to help the project survive in the difficult few weeks after Dr. Shorey was killed. I am also grateful for the optimism, enthusiasm and organizational efforts of Albert Keck of Hadley Date Gardens, and acknowledge the considerable in-kind contributions in test sites, equipment, and labor made by the cooperating growers

and packers associated with this project. I thank the Department of Pesticide Regulation for financial support for this project.

Figure 1. Experimental plot layouts.

1. Hadley Ranch plot layout (40 acres). Black dots are trap locations



2. Rancho Eileen plot layout (160 acres). Black dots are trap locations. Dotted lines are dirt roads.

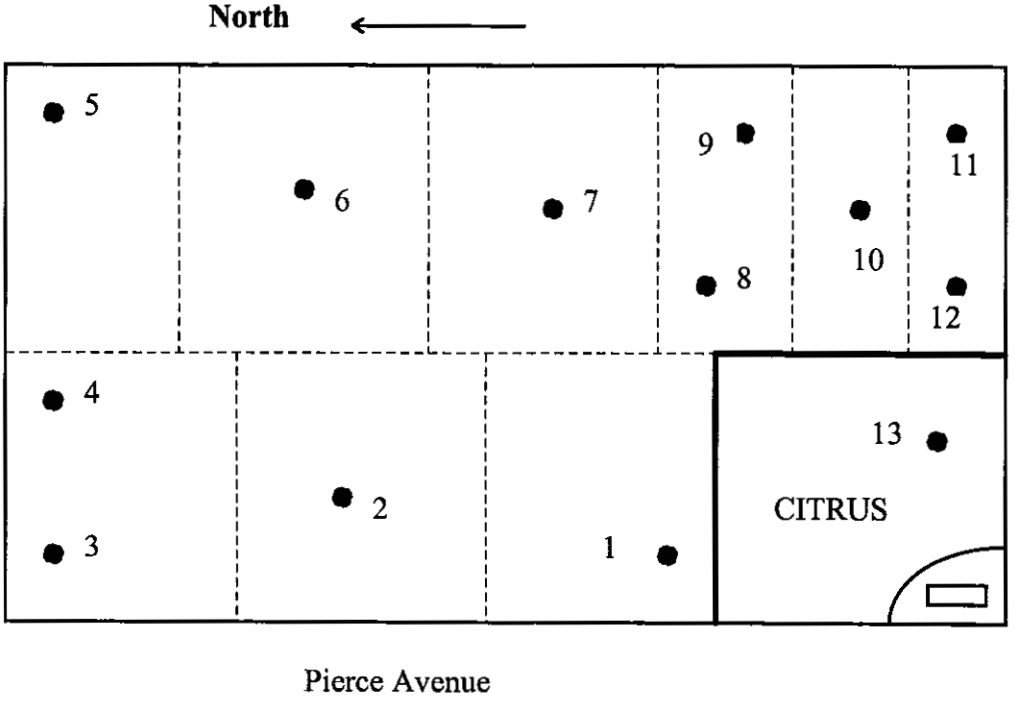


Figure 2. Mean weekly trap catches of carob moths from Hadley Ranch, 1998 (n=6), and from 7 untreated control sites.

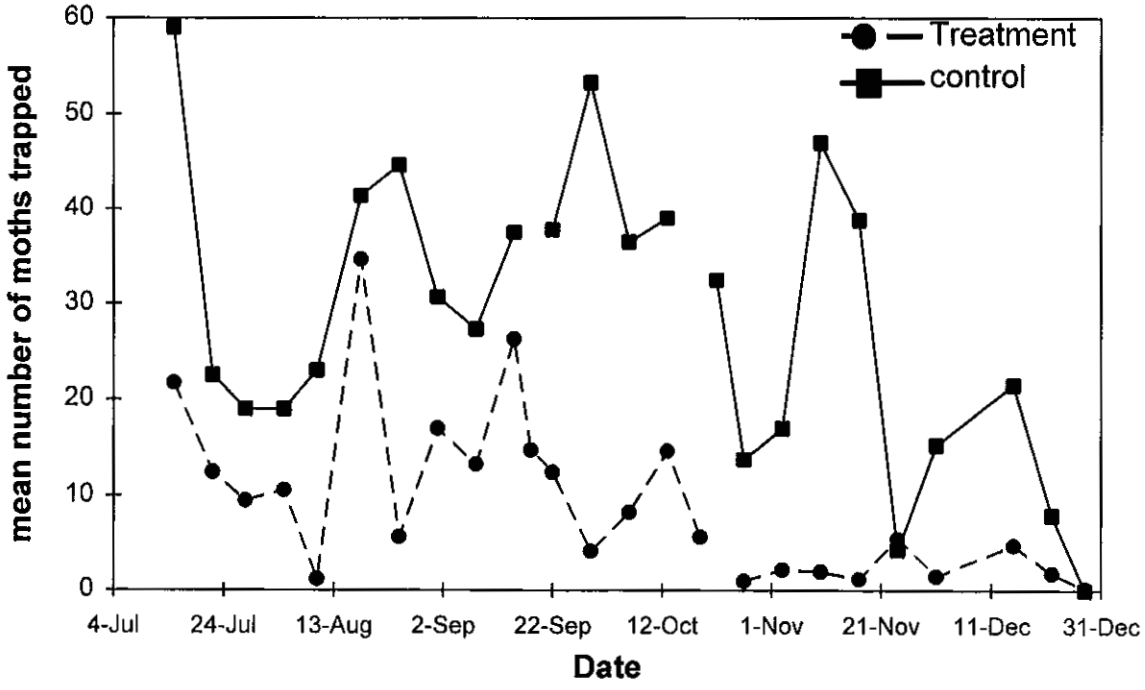


Figure 3. Mean weekly trap catches of male carob moths at Rancho Eileen, 1998 (n=12).